Article

Evaluation of the sterility of single-dose medications used in a multiple-dose fashion

Elizabeth P. Martin, Jean Mukherjee, Claire R. Sharp, Virginia B. Sinnott-Stutzman

Abstract — Bacterial proliferation was evaluated in single-dose medications used in a multi-dose fashion and when medications were intentionally inoculated with bacteria. Of 5 experimentally punctured medications, 1 of 75 vials (50% dextrose) became contaminated. When intentionally inoculated, hydroxyethyl starch and heparinized saline supported microbial growth. Based on these findings, it is recommended that hydroxyethyl starch and heparinized saline not be used in a multi-dose fashion.

Résumé — Évaluation de la stérilité des médicaments à dose unique utilisés pour plusieurs doses. On a évalué la prolifération bactérienne dans les médicaments à dose unique utilisés pour plusieurs doses et lorsque les médicaments sont intentionnellement inoculés avec des bactéries. Parmi les cinq médicaments ayant subi une ponction expérimentale, 1 des 75 flacons (50 % dextrose) a été contaminé. Lorsqu'ils étaient inoculés intentionnellement, l'hydroxyéthylcellulose et le soluté physiologique hépariné supportaient la croissance microbienne. En se basant sur ces résultats, il est recommandé que l'hydroxyéthylcellulose et le soluté physiologique hépariné ne soient pas utilisés pour plusieurs doses.

(Traduit par Isabelle Vallières)

Can Vet J 2017;58:1187–1190

Introduction

Many medications manufactured for human patients are used in an off-label manner in the veterinary field. Medications labeled as single dose vials that are commonly used in a multiple dose fashion in veterinary hospitals are of concern (1). Single dose vials lack antimicrobial preservative and are manufactured to be used only as a single dose administered to a single patient (2–4). By contrast, multiple dose vials typically contain an antimicrobial preservative or have antimicrobial properties, such as high osmolarity, designed to inhibit proliferation of contaminating bacteria that might enter with multiple punctures (2,3). Because it is against the safe injection practices of the Centers for Disease Control and Prevention and the World Health Organization to use single dose vials in a multi-dose fashion in humans, there are no prospective studies that have evaluated whether this practice places veterinary patients at risk for infection (2–4).

Reports of disease transmission in humans from improper injection practices using single dose and multi-dose vials appear periodically in public health literature (5–10). Point-prevalence studies evaluating contamination of multi-dose vials in human hospitals have revealed contamination rates of 0.9% to 5.6% (5,6). At a veterinary teaching hospital, the multi-dose vial contamination rate was 18%, with most growth occurring in preservative-free saline (single dose vial) being used to dilute medications before administration (1). It is therefore vital to investigate whether using single dose vial medications in a multi-dose fashion will result in microbial contamination.

This investigation was designed to evaluate single dose vial medications commonly used in a veterinary emergency setting in an off-label, multi-dose fashion. The first objective of the study was to prospectively evaluate the contamination of single dose vials used in a multi-dose fashion under simulated clinical conditions, and to determine whether the medications under study would support bacterial proliferation over time. The second objective was to determine whether single dose vials intentionally inoculated with bacteria would support bacterial proliferation over time. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the bacteria that were selected because

Cape Cod Veterinary Specialists, 11 Bourne Bridge Approach, Buzzards Bay, Massachusetts 02532, USA (Martin); Tufts University Cummings School of Veterinary Medicine, 200 Westboro Road, North Grafton, Massachusetts 01536, USA (Mukherjee); College of Veterinary Medicine, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia 6150 (Sharp); Angell Animal Medical Center, 350 S Huntington Avenue, Boston, Massachusetts 02130, USA (Sinnott-Stutzman).

Address all correspondence to Dr. Elizabeth Martin; e-mail: epmartin.vmd@gmail.com

A portion of this investigation was presented in abstract form at the International Veterinary Emergency and Critical Care Symposium in 2014.

Table 1. Characteristics of the fluids evaluated in this study relative to canine plasma.

		Fluid characteristics		
Fluid type	pH	Osmolarity (mOsm/L)	Buffer	Additional ingredients
Canine plasma	7.4	290–310 mOsm/L	n/a	n/a
6% Hydroxyethylstarch[a]	5.9	309 mOsm/L	none	Na: 154 mEq/L Cl: 154 mEq/L
Mannitol[b]	4.5–7.0	1098 mOsm/L 1100 mOsm/L	none	n/a
50% Dextrose[c]	3.5–6.5	2525 mOsm/L	none	n/a
Heparin[d]	5.0–7.0	287 mOsm/L	none	n/a
0.9% NaCl[e]	5.5	308 mOsm/L	none	Na: 154 mEq/L Cl: 154 mEq/L
Hypertonic saline 7.2%[f]	5.0	2464 mOsm/L	none	Na: 1232 mEq/L Cl: 1232 mEq/L

Data from Product Information Sheets for [a] HESpan; [b] Mannitol 20%; [c] Dextrose 50%; [d] Heparin; [e] 0.9% NaCl; [f] 7.2% Hypertonic saline. n/a — Not available.

of their importance as nosocomial pathogens (11,12,13). We hypothesized that certain single dose vial medications would not support bacterial growth, despite use in multi-dose fashion and intentional inoculation.

Materials and methods

Medications

The following medications were evaluated: 6% hydroxyethylstarch in 0.9% NaCl (Hespan; Braun, Irvine, California, USA), 20% mannitol in water (Mannitol Injection 20%; NeoGenVet, Lexington, Kentucky, USA), 50% dextrose in water (Dextrose 50% Injection; VetOne, MWI, Boise, Idaho, USA), 7.2% hypertonic saline (Equi-Phar Equine 7 HSS; VEDCO, St. Joseph, Missouri, USA), and 10 U/mL heparinized 0.9% saline (0.9% Sodium Chloride Injection, USP; Hospira, Lake Forest, Illinois, USA; Heparin Sodium Injection, Sagent Pharmaceuticals, Schaumburg, Illinois, USA) (Table 1). Each medication container had a rubber bung for introduction of a hypodermic needle and withdrawal of fluid. All study medications were stored on open display in the triage area of a private practice, emergency and specialty veterinary hospital during the course of the study to most closely mimic how open single dose vials are often stored in an emergency room. Medications were clearly labeled as study medications to avoid accidental clinical use.

Experimental puncture study

Fifteen containers of each medication (6% hydroxyethylstarch in 0.9% NaCl, 20% mannitol in water, 50% dextrose in water, 7.2% hypertonic saline, and 10 U/mL heparinized 0.9% saline) were divided into 3 groups of 5 containers each based on puncture frequency — 5 punctures/day, 1 puncture/day, and 1 puncture/week. This puncture schedule was designed to mimic frequent, moderate, and infrequent use, respectively.

A veterinary technician was randomly chosen to perform the medication container punctures beginning on Day 0 through Day 27. Randomization was accomplished by non-algorithmic means (drawing sealed envelopes) with no limitation on the frequency with which a technician could be chosen. Technicians were provided with 22-gauge (G) needles attached to 3-mL syringes (Nipro Medical Corporation, Miami, Florida, USA) for each puncture and instructed to withdraw 0.2 mL of medication per puncture. The only guidance was on how many punctures to perform.

No further procedures were conducted with the withdrawn medication fluid. On Day 0, before the start of the puncture schedule by the technicians and then on Days 1, 7, 14, and 28, a designated investigator (EPM) removed 1 mL of medication for individual culture using a sterile 3-mL syringe with an attached 22-G needle and then transferred each sample into a 3-mL sterile plain red top tube (BD Vacutainer). Samples were shipped overnight on icepacks to a commercial veterinary microbiology laboratory (IDEXX Laboratories, North Grafton, Massachusetts, USA) for quantitative aerobic culture and microbial identification. A 100 µL volume of each sample was streaked onto a Tryptic Soy Agar plate containing 5% sheep blood and a MacConkey agar plate and incubated at 37°C in the presence and absence of 5% CO_2, respectively. The plates were examined for growth after 24 and 48 h. If there was bacterial growth, the number of colony-forming units (CFU) was determined and isolated organisms were sent for identification using the Vitek XL system (BioMérieux, Marcy-l'Étoile, France). When automated processes failed to identify an organism or the identification was suspected to be incorrect, standard biochemical reactions and colony morphology were used for identification.

Saline flush syringes

On Day 0, the randomly selected technician prepared heparinized flush syringes by removing 1 mL of 10 000 U/mL heparin from a new bottle and injected it into a 1 L bag of 0.9% NaCl. The technician then used the resulting solution to make twenty 3-mL flushes using 3-mL syringes with attached 22-G needles. The 20 syringes were divided into 4 groups of 5; each set of 5 syringes was assigned to be cultured on Days 1, 7, 14, or 28. On Day 0, 1 mL of the newly heparinized saline was removed and a 100 µL sample was cultured immediately to check for sterility. On Days 1, 7, 14, and 28, 0.2 mL was

withdrawn for culture from each of 5 syringes within the designated group and pooled into a sterile 3-mL red top tube (BD Vacutainer).

Samples were shipped overnight on icepacks to IDEXX Laboratories, North Grafton, Massachusetts, for quantitative aerobic culture and microbial identification as described for the experimental puncture study.

Intentional medication contamination

To simulate low-level contamination, likely to occur in a clinical setting, 5 containers of each of the 5 medications were intentionally inoculated on Day 0 with 2 strains of bacteria each — *Staphylococcus aureus* (strain ATCC 6538) and *Pseudomonas aeruginosa* (strain ATCC 9027). Bacteria were reconstituted as directed by the manufacturer (Quanti-Cult Plus Remel Microbiology Products; Lenexa, Kansas, USA). Based on the manufacturer's quantitation, approximately 300 CFUs of each bacterial species was inoculated into each medication container. Initial estimated concentrations for each bacterial species were 6 CFU/mL 50% dextrose, 3 CFU/mL 20% mannitol, 0.6 CFU/mL 6% hydroxyethylstarch, 0.3 CFU/mL for heparinized saline, and 0.3 CFU/mL for 7.2% hypertonic saline. A veterinary technician was randomly chosen to perform medication container punctures 5 times each day as described for the experimental puncture study beginning on Day 0 and ending on Day 27.

On Day 0, immediately prior to intentional contamination and initiation of the puncture schedule by the technicians, and then on Days 1, 7, 14, and 28, a designated investigator (EPM) removed 0.2 mL of medication for culture from each of the 5 containers within a designated group. Medication withdrawn from containers within a group was pooled in a 3-mL sterile plain red top tube (BD Vacutainer), then shipped and subjected to microbial culture and quantification of isolated bacteria as described for the experimental puncture study.

A follow-up study was conducted using high levels of each bacterial strain to evaluate whether the medications selected supported or inhibited bacterial growth. Two containers of each of the 5 medications were intentionally inoculated on Day 0 with *S. aureus* (ATCC 6538) or *P. aeruginosa* (ATCC 9027). The 2 bacterial strains (Culti Loops; Remel Microbiology Products) were subcultured multiple times and single colonies were used to inoculate ~25 mL sterile brain heart infusion (BHI) broth (Difco, Becton Dickinson, Sparks, Maryland, USA). The absorbance at 600 nm (A_{600}) was determined following incubation with shaking at 37°C for ~6 h. Log-phase cultures were used to prepare serial dilutions of each bacterial strain and each medication container was inoculated on Day 0 with either *S. aureus* (ATCC 6538) or *P. aeruginosa* (ATCC 9027) to achieve a final concentration of 1000 CFU/mL. On Day 0, immediately before and immediately after intentional contamination, and subsequently on Days 1, 2, 3, 4, 5, and 6, 0.5 mL of liquid was removed from each medication container and serially diluted 1:5 in sterile PBS across a 12-well dilution boat. A 200-μL volume of the undiluted sample and each serial dilution was spread onto separate Columbia agar plates containing 5% sheep blood (Remel). Colonies were counted after overnight incubation at 37°C, and CFU/mL were determined for each sample at each time point.

Statistical methods

This investigation was designed to detect single dose vial medications with a high risk of contamination. Assuming 100% recovery of viable organisms if contamination and proliferation above the limit of detection occurred at any time point, this investigation was powered to have a 95% chance of detecting a 45% contamination rate. As the incidence of positive cultures was extremely low, statistical methods to determine relative risk of contamination were not carried out.

Results

In the experimental puncture study, *Micrococcus luteus* (< 100 CFU/mL) was isolated on culture Day 7 from a single container of 50% Dextrose that had been punctured once weekly. No other test medications that underwent experimental puncture were positive for bacterial growth throughout the study.

Following intentional inoculation with low doses of *P. aeruginosa* and *S. aureus,* only *P. aeruginosa* was isolated from 6% hydroxyethylstarch and heparinized 0.9% saline. Growth was first detected on Day 7, increased by Day 14, and then decreased by Day 28.

After intentional inoculation with *P. aeruginosa* at 1000 CFU/mL, marked growth was observed in heparinized 0.9% saline (6.3×10^3 CFU/mL by Day 6). Sustained moderate growth was also observed in 6% hydroxyethylstarch. Growth was also observed in 20% mannitol and in 7.2% hypertonic saline; however, this was no longer evident by Days 4 and 6, respectively. No growth was observed in 50% dextrose.

The bacteria persisted for variable lengths of time after intentional inoculation with *S. aureus* at 1000 CFU/mL, but no proliferation was observed.

Discussion

This study sought to determine whether commonly used medications could become contaminated and sustain bacterial growth. Single dose vial medications of 6% hydroxyethylstarch in 0.9% NaCl, 20% mannitol in water, 50% dextrose in water, 7.2% hypertonic saline, and 10 U/mL heparinized 0.9% saline were chosen because they are commonly used in veterinary emergency hospitals. These vial medications represent a wide spectrum of pH and tonicity (Table 1), which may impact each medication's inherent ability to sustain bacterial growth. The first part of this study examined the potential for contamination when medications were experimentally punctured multiple times per day or week. The second part of this study examined the potential for microbial growth following intentional contamination.

Following experimental puncture, 1 to 5 times/day or once each week over a 28-day period, a single vial of 50% dextrose yielded a positive aerobic bacterial culture of *M. luteus,* with no subsequent growth noted throughout this period of time. *Micrococcus luteus* is typically a non-pathogenic, Gram-positive human skin commensal, although clinical infections may occur in immune-compromised individuals (14). A transient positive

culture with subsequent clearing of the inoculum suggests 50% dextrose is hostile to microbial growth, but whether it meets USP or British Pharmacopeia standards for antibacterial properties is beyond the scope of this investigation.

Following intentional inoculation with 300 CFU *P. aeruginosa* and *S. aureus,* both 6% HES and heparinized saline supported a 1000× increase in *P. aeruginosa.* This suggests that even if a relatively minute contamination occurs, bacterial proliferation may continue unopposed by any inherent property of the solution and use as multi-dose vials could result in iatrogenic bacteremia.

It is unclear why some single dose vial medications supported microbial growth when intentionally contaminated while others did not. Of particular interest are the medications that did not support proliferation. Interestingly, these are all solutions that are hyperosmolar to canine plasma. While osmotic tolerance mechanisms exist among select bacterial species, several prior investigations have revealed that hyperosmolar solutions can reduce bacterial survival (15–17). Further investigation into the effects and mechanisms of osmolarity, as well as other chemical properties of medications which may prove hostile to bacterial growth, would aid in our understanding of the risk for bacterial contamination of preservative-free medications used in a multi-dose fashion. Examples of potentially impactful chemical properties include pH, carbohydrate source, buffering solution and solution viscosity.

Overall, this investigation revealed that accidental contamination events could occur when single dose vials were used in a multiple dose fashion in controlled, experimental conditions, and that microbial proliferation was possible with the introduction of a small bacterial inoculum into certain medications. Based on the ability of 6% hydroxyethylstarch and heparinized 0.9% saline to support proliferation of *P. aeruginosa* when intentionally inoculated even with low levels of bacteria (< 10 CFU/mL), the use of these medications in a multi-dose fashion should be considered a patient safety risk. Although the other medications did not support bacterial proliferation when inoculated with low levels of bacteria, it would be inappropriate to infer that the use of preservative-free 50% dextrose, hypertonic saline, or mannitol in a multiple dose fashion is safe, given that our initial inoculum was below the limits of detection for this portion of the study. A high contamination rate selected in our power analysis, to eliminate drugs for consideration as multi-dose vials, was not meant to definitively identify safe drugs.

Inoculation with a high level of bacteria (1000 CFU/mL) revealed that all of these medications, with the exception of 50% dextrose, can support persistence and/or growth of *P. aeruginosa* and *S. aureus* for some time. Limitations of the study are that the number of medications and organisms evaluated was limited and that bacterial culture is a relatively insensitive technique. However, the results suggest that further investigation into contamination of medications is warranted, particularly in reference to multiple punctures over a period of time and length of storage following initial entry.

Acknowledgments

We thank Alexa Foss for her expert laboratory assistance, and Kiko Bracker DVM, DACVECC for the initial idea for this project. Most of the funding was through the 2013 ACVECC research grant with additional funding provided by Cape Cod Veterinary Specialists. CVJ

References

1. Sabino C, Weese J. Contamination of multiple-dose vials in a veterinary hospital. Can Vet J 2006;47:779–782.
2. Centers for Disease Control and Prevention. CDCs Position: Protect Patients Against Preventable Harm from Improper Use of Single-Dose/Single-Use Vials. 2012. Available from: http://www.cdc.gov/injectionsafety/CDCposition-SingleUseVial.html Updated: August 30, 2016. Last accessed September 5, 2017.
3. Centers for Disease Control and Prevention. Questions about Single-Dose/Single-Use Vials. 2011. Available from: http://www.cdc.gov/injectionsafety/providers/provider_faqs_singlevials.html Updated: February 9, 2011. Last accessed September 5, 2017.
4. Best Practices for Injections and Related Procedures Toolkit. Geneva, World Health Organization, 2010. Available from: http://whqlibdoc.who.int/publications/2010/9789241599252_eng.pdf Last accessed September 5, 2014.
5. Mattner F, Gastmeier P. Bacterial contamination of multi-dose vials: A prevalence study. Am J Infect Control 2004;32:12–16.
6. Motamedifar M, Askarian M. The prevalence of multi-dose vial contamination by aerobic bacteria in a major teaching hospital, Shiraz, Iran, 2006. Am J Infect Control 2009;37:773–777.
7. Dolan S, Felizardo G, Barnes S, et al. APIC Position Paper: Safe injection, infusion and medication vial practices in healthcare. Am J Infect Control 2010;38:167–172.
8. Anderson S, Rigler J, Oberoi V, et al. Invasive *Staphylococcus aureus* infections associated with pain injections and reuse of single-dose vials — Arizona and Delaware, 2012. Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report. 2012;61:501–504. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6127a1.htm?s_cid=mm6127a1_w Last accessed September 5, 2017.
9. Machikanti L, Falco F, Benyamin R, et al. Assessment of infection control practices for interventional techniques: A best evidence synthesis of safe injection practices and use of single-dose medication vials. Pain Physician 2012;15:E573–614.
10. Cohen A, Ripath A, Noble-Wang J, et al. Outbreak of *Serratia marcescens* bloodstream and central nervous system infections after interventional pain management procedures. Clin J Pain 2008;24:374–380.
11. Kerr KG, Snelling AM. *Pseudomonas aeruginosa:* A formidable and ever-present adversary. J Hosp Infect 2009;72:338–344.
12. Agarwal L, Singh AK, Sengupta C, Agarwal A. Nasal carriage of methicillin- and mupirocin-resistant *S. aureus* among health care workers in a tertiary care hospital. J Res Pharm Pract 2015;4:182–186.
13. Sydnor E, Perl T. Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev 2011:141–173.
14. Bannerman TL, Peacock SJ. *Staphylococcus, Micrococcus,* and other catalase-positive cocci. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Faller MA, eds. Manual of Clinical Microbiology. Vol 1. 9th ed. Washington, DC: ASM Press, 2006:390–411.
15. Lynch T, Morris T, Dix J, Nelson P. Bacterial counts in canine duodenal fluid after exposure to saline, sodium bicarbonate and hypertonic dextrose solutions used to maintain patency of chronically implanted catheters. Lab Anim 1999;33:143–148.
16. Sleator RD, Hill C. Bacterial osmo-adaptation: The role of osmolytes in bacterial stress and virulence. FEMS Microbiol Rev 2002;26:49–71.
17. Barraud N, Buson A, Iarolimek W, Rice S. Mannitol enhances antibiotic sensitivity of persister bacteria in *Pseudomonas aeruginosa* biofilms. PLOS One 2013;8:e84220.